

## **Remarks**

### **I. Rejections Under 35 U.S.C. § 112, Second Paragraph**

#### **A. Basis for the rejection**

Claims 5 and 13 were rejected under 35 U.S.C. § 112, second paragraph as indefinite. Office Action, page 2. The Patent Office states that the phrase "about 12 hours, about 24 hours or about 48 hours" appearing in claims 5 and 13 render the claims indefinite because as the upper and lower limits of the recited time periods are not clear. The Patent Office also states that the phrase "at a temperature of about 4 °C, about 10 °C, about 15 °C, about 20 °C or about 25 °C" further renders claim 13 indefinite as it is unclear whether the applicants intended to claim the recited time periods and/or the recited temperatures in alternate forms. The Examiner suggesting insert the word "and" between the terms "48 hours" and "at a temperature in claim 13.

#### **2. Applicants' response**

Claim 13 has been amended as suggested by the Examiner. Claims 5 and 13 have also been amended to recite time and temperature ranges, thereby clearly defining the upper and lower limits of the recited time periods and temperatures. Applicants believe that these amendments overcome this rejection under § 112, second paragraph, and respectfully request that it be withdrawn.

### **II. Prior Art Rejections**

#### **A. Rejection over Brochner et al.**

##### **1. Basis for the rejection**

Claims 1-6, 9-14 and 16-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Bochner et al., U.S. Patent No. 4,460,262. The Patent Office characterizes the Bochner reference as teaching a method for the preparation of hGH from transformed *E. coli* comprising the steps of culturing a transformant of *E. coli* in 500 mL LB and O tetracycline at 37 °C for about 8 hours (during which hGH is secreted into the periplasm and the transformed cells are concentrated due to growth), followed by seeding 500 mL the inoculum culture into a 10L fermenter containing phosphate-limiting medium at 37 °C and pH 7.5 for about 36 hours (which the Patent Office characterizes as "about 24 hours or about 48 hours"), after which 1-butanol is added to the fermenter and steam is immediately injected into the fermenter jacket so that the temperature of the tank rises rapidly to 50 °C, and is held at this temperature for 10 minutes. The cells are then frozen until further processing is required. According to the Patent Office, "any of the steps following fermenting the transformant bacteria in 500 mL culture medium is

considered to be an interrupting step.” Office Action, page 4. The Patent Office concludes that “the teachings of Bochner et al meet every limitation of the instant broadly [sic] claims.” *Id.*

## 2. Applicants' response

The applicants respectfully submit that the Patent Office has misinterpreted the teachings of the Bochner reference as they relate to the present claims. Specifically, the Patent Office has erred in characterizing *any* of the steps following the fermentation in 500 mL of medium as an “interrupting” step within the meaning of the present claims. The present specification expressly defines the meaning of the term “fermentation” as it relates to the claimed invention:

The fermentation of the polypeptide is performed in a fermentation medium under appropriate conditions such that the polypeptide is secreted into the periplasm of the host cell (described e.g. in Skerra and Plückthun, *Science* 240, 1038-1041, 1988).

As used herein, the term “fermentation” is understood to mean growing a host cell in an appropriate fermentation medium for an appropriate period of time under appropriate conditions such that the polypeptide is produced by the host cell and secreted into the periplasm.

Specification, page 6, 6<sup>th</sup> full paragraph and paragraph bridging pages 6 and 7. Thus, the specification clearly and expressly defines “fermentation” as the phase where cells are growing, producing the heterologous protein, and secreting it into the periplasm. Conversely, “interruption” is clearly and expressly stated to be a period of time occurring *after* completion of fermentation, and before further processing of the fermentation harvest broth:

In the context of the present invention it has surprisingly been found that the yield of a recombinant polypeptide expressed in a bacterial host comprising a periplasm can be increased by *interrupting* the isolation process *after fermentation* and put[ing] on hold *further processing* of the fermentation harvest broth *before the subsequent steps of extraction and isolation* of said polypeptide are performed.

Specification, page 4, 7<sup>th</sup> full paragraph. It is therefore abundantly clear from the specification that the interruption period of the presently claimed methods occurs *after* the phase of cell growth and expression/secretion of the heterologous polypeptide, and *before* the steps of extraction and isolation of the heterologous polypeptide.

With the foregoing understanding of the claimed invention, it is seen that the Bochner et al does not, in fact, teach each and every element of present claims 1-6, 9-14 and 16-22. Specifically, Bochner et al do not teach a method comprising an interruption between fermentation and further processing of the harvest broth of any significant length of time.<sup>1</sup>

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<sup>1</sup> Obviously, even in the methods of the prior art there always are a few minutes of time between harvesting the fermentation broth and the further processing steps of killing the cells and extracting the expressed protein, simply to handle the material in passing from one step to the next. These delays can be considered insignificant because they are not a designed part of the process.

Bochner et al. expressly state that in their method “[p]referably the cells are permitted to accumulate periplasmic heterologous protein to a maximum level, usually during a growth stage following logarithmic growth *prior to initiation of the treatment provided herein.*” Col. 4, lines 41-45 (emphasis added). According to the teachings of Bochner, “[t]he cells should be killed as soon as this point is reached or shortly thereafter.” Col. 4, lines 45-46. It is *after* the cells have been fermented and killed that the harvest broth is processed to recover the heterologous protein from the periplasm. Therefore, the “fermentation” period taught by Brochner et al. is the entire period of cell growth and accumulation of the heterologous polypeptide in the periplasm, terminated by killing the cultured cells. This is indeed the procedure followed in Example 8, cited by the Patent Office. In Example 8, the 500 mL inoculum culture is incubated for 8 hours to increase the cell titre of the culture, then immediately seeded into the large-volume 10 L fermentation vessel. Col. 12, lines 3-14. Though there is a medium change in the 10 L vessel, the cells are actively growing, and the entire process is “fermentation,” as in fact it is characterized by Bochner et al.: “[t]hirty-six hours after the *start of fermentation* the cells are killed and harvested.” Col. 12, lines 25-26. Throughout this fermentation period, the cultured cells are kept in fermentation medium and under conditions such that the heterologous polypeptide is secreted into the periplasm. After the cells have been killed, *ending the fermentation stage of the method*, they are then frozen for storage. The processing of the stored cells to extract and isolate the heterologous polypeptide begins by thawing the frozen cell paste obtained at the end of the fermentation step, resuspending the cells in buffer, and gently stirring them at about 4 °C for a period of between 10 and 60 minutes, preferably for 30 minutes. Col. 5, lines 53-66. During this step of the Brochner process, the cells are not in fermentation medium. After this 10-60 minute period of stirring, the suspended cells are processed further to extract and isolate the heterologous protein from the periplasm. Thus, contrary to the Patent Office’s interpretation of the teachings of Bochner et al., the 10-60 minute time period during which the cells are held does not fall within the meaning of the term “interrupting further processing,” as used in present claims 1-6, 9-14 and 16-22.

In view of the foregoing, the applicants submit that this rejection of claims 1-6, 9-4 and 16-22 anticipation by the Brochner et al. can properly be withdrawn. Reconsideration and withdrawal of the rejection is respectfully requested.

#### **B. Rejection over Hauptmann et al.**

Claims 1, 6-8, 13-14 and 16-21 were rejected under 35 U.S.C. § 102(b) as being anticipated by Hauptmann et al., U.S. Patent No. 5,710,027. The Patent Office characterizes the Hauptmann et al. reference as teaching a process for preparing human IFN $\alpha$ 2c by recombinant expression and secretion of the protein into the periplasmic space of *E. coli*. Office Action, page 4. According to the Patent Office, Hauptmann et al. teaches that prior to the extraction step, the fermentation mixture is cooled down to about 10 °C, the pH adjusted to 2.0, and the biomass separated by centrifugation and frozen at -70 °C. Office Action, pages 4-5.

According to the Patent Office, these teachings meet each and every limitation of the claims. Office Action, page 5.

## **2. Applicants' response**

The teachings of the Hauptmann et al. reference focus almost entirely on only two aspects in the protein production process: preparation of an expression vector which permits "an efficient and stable system for the expression/secretion of the protein into the periplasmic space or culture medium," and "a process for highly purifying the expressed protein gently, without the need for immunoaffinity chromatography." See, e.g., col. 2, lines 33-42. There is no teaching, general or specific, of including an interrupting period within the meaning of the term "interrupting further processing," as used in present claims, after the fermentation step, and prior to the extraction step. The example cited by the Patent Office, Example 2, only covers the fermentation stage of the process, which was ended by inactivating the biomass mixture by cooling and adjusting the pH, and separating the biomass from the fermentation medium by centrifugation. Col. 13, lines 61-67. In Example 3, the inactivated biomass was suspended in 500 ml of 1% acetic acid, then stirred for 1 hour at 0 °C, after which polyethylenimine was added and the suspension was stirred for an additional 2 hours. IFNa2c was extracted by centrifugation, with an average extraction yield of only 29.3±5.9%. Col. 14, lines 5-16.

The 3 hour period described in Example 3 of Hauptmann et al. is *not* an interrupting period within the meaning of present claims 1, 6-8, 13-14 and 16-21, but is rather the extraction step of the purification process. This is made clear by the fact that the fermentation medium was entirely removed prior to this step, and replaced with extraction solvents, such that after this 3 hour period removal of the bacteria by centrifugation yielded directly the crude INFα2c extract that was subjected to the chromatographic purification process exemplified in the subsequent Examples. The express language of present claims 1, 6-8, 13-14 and 16-21 states that the interrupting step comprised maintaining *the fermentation harvest broth* under defined conditions of temperature and pH *prior to extraction*.

In view of the foregoing, the applicants respectfully submit that Hauptmann et al. does not teach each and every limitation of present claims 1, 6-8, 13-14 and 16-21, expressly or inherently, and thus does not anticipate those claims. Reconsideration and withdrawal of this rejection is respectfully requested.

## **C. Rejection over Hart et al.**

### **1. Basis for the rejection**

Claims 1-3, 6-9, 13-14 and 16-22 were rejected under 35 U.S.C. 102(b) as being anticipated by Hart et al. (Bio/Technology 12:1113-1117, 1994). Office Action, page 5. The Patent Office states that

Hart et al. discloses a new method for *in situ* isolation of periplasmic human IGF-I from recombinant *E.coli*, said method comprises the *in situ* solubilization procedure entailing the steps of

adjusting the pH of the fermentation broth to pH10 and the addition of urea and DTT to a respective final concentration of 2M and 10 MM; followed by an incubation at 37 °C for about 1 hour, then cooled to 22 °C, about 20 °C (see at least the abstract and particularly page 1116, right column, first two full paragraphs). The *in situ* solubilization procedure is an interrupting procedure prior to the aqueous two-phase extraction.

*Id.* The Patent Office concludes that the teachings of Hart et al. meet all the limitations of the instant claims, and therefore anticipates them.

### 1. Applicants' response

The applicants respectfully submit that the Patent Office has misconstrued Hart et al. as it applies to the present claims. The present claims recite an "interrupting" period between fermentation and the further processing of the fermentation harvest broth. In other words, the present claims recite a method wherein the harvest broth is held for an extended period of time<sup>2</sup> under defined conditions of temperature and pH, after fermentation is complete, and *prior* to carrying out the further processing of the fermentation harvest broth. In contrast, Hart et al. teach a fairly typical process wherein cells are fermented, fermentation is halted, and the fermentation broth is immediately subjected to processing/extraction steps – in this case the *in situ* solubilization of the expressed protein, followed immediately by two-phase extraction and precipitation. The one hour period of time that the Patent Office characterizes as an "interrupting procedure" is in fact the first of a series of three processing steps – solubilization, extraction and precipitation. Hart et al. in fact refers to the solubilizing step along with the extraction step as a part of the method for recovering the expressed protein:

**Recovery yield did not depend on the order of reagent addition. IFG-I solubilization and extraction was typically complete in 1 hour.** (page 1114, left column, first full paragraph) (emphasis added).

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**"In situ solubilization followed by aqueous two-phase extraction provides a method for recovering all recombinant protein regardless of its initial solubility.** (page 1115, left column, first full paragraph) (emphasis added).

This one-hour solubilization/extraction step taught by Hart et al. does not fall within the meaning of "interrupting further processing" as used in present claims 1-3, 6-9, 13-14 and 16-22, because it is for the purpose, and has the effect, of processing the fermentation harvest broth to recover the expressed protein.

In view of the foregoing, the applicants respectfully submit that Hart et al. does not teach every limitation of present claims 1-3, 6-9, 13-14 and 16-22, expressly or inherently, and thus does not anticipate those claims. Reconsideration and withdrawal of this rejection is respectfully requested.

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<sup>2</sup> See footnote 1, above.

## **D. Rejection over Kwon et al.**

### **1. Basis for the rejection**

Claims 1, 6-15 and 17-22 were rejected under 35 U.S.C. § 102(e) as being anticipated by Kwon et al., U.S. Publication No. 2004/0151695). The Patent Office has characterized the Kwon et al. reference as disclosing a method for producing human interferon alpha, including human interferon alpha b, by recombinantly expressing and secreting the same in the periplasm of a genetically modified *E.coli*. Office Action, page 6. According to the Patent Office, Kwon et al. teaches a process whereby a 3 hour induction period is followed by a 20 minute centrifugation to precipitate bacterial cells, resuspension of the cells in an isotonic solution which is allowed to stand at room temperature for 30 minutes, after which bacterial cells are collected by centrifugation. *Id.* The Patent Office concludes that because these steps occur prior to resuspension of the collected cells to extract the proteins present in the periplasm, they constitute an interrupting step, and therefore the teachings of Kwon anticipate the rejected claims.

### **2. Applicants' response**

The present claims recite an "interrupting" period between fermentation and the further processing of the fermentation harvest broth (in claim 13 a period of specifically about 12 to about 48 hours). In other words, the present claims recite a method wherein the harvest broth is held for a certain period of time<sup>3</sup> under defined conditions of temperature and pH, *after* fermentation is complete, and *prior* to carrying out the further processing of the fermentation harvest broth. In the Kwon et al. process, the 30 minute standing period referred to by the Patent Office is part of the extraction process: the cultures are harvested after fermentation, centrifuged to concentrate the cells, the cells were then immediately disrupted by subjected to osmotic shock, then immediately suspended in an isotonic solution and allowed to stand for 30 minutes (not in the fermentation harvest broth), then immediately extracted to recover the expressed protein. Present claims 1, 6-15 and 17-22 recite interruption of processing *the fermentation broth*, not resuspended cells, *prior* to processing steps. Furthermore, as explained in the specification, methods such as that taught by in Kwon et al. result in significant contamination of the recovered protein solution/suspension, and significant reductions in yield. See, e.g., pages 1-2, bridging paragraph, page 2, 1<sup>st</sup> full paragraph, page 4, 1<sup>st</sup> through 3<sup>rd</sup> full paragraphs.

Because the 30 minute standing period taught in Kwon et al. is a part of the processing of the fermentation broth to recover the expressed protein, as evidenced by, for example, the fact that it is carried out in an isotonic solution of precipitated cells and not in fermentation harvest broth, it does not fall within the meaning of "interrupting further processing" as used in present claims 1, 6-15 and 17-22.

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<sup>3</sup> See footnote 1, above.

In view of the foregoing, the applicants respectfully submit that Kwon et al. does not teach every limitation of present claims 1, 6-15 and 17-22, expressly or inherently, and thus does not anticipate those claims. Reconsideration and withdrawal of this rejection is respectfully requested.

#### CONCLUSION

In view of the foregoing, the applicants respectfully submit that the present claims are not anticipated by any of the cited prior art, and are in good condition for allowance. Favorable action on the claims is earnestly solicited.

Respectfully submitted,



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